Chapter 14
Essential Fatty Acids

Esther Granot and Richard J. Deckelbaum

Keywords Essential fatty acids · DHA · EPA · Arachidonic acid (AA) · Alpha-linolenic acid (ALA) · Polyunsaturated fatty acids

Learning Objectives

- Review the role that essential fatty acids should have as part of nutrition interventions for low- and middle-income countries
- Identify the dietary sources for essential fatty acids and their conversion to other n-3 and n-6 fatty acids
- Describe how essential fatty acids are associated with birth outcomes
- Explain the mechanisms for how essential fatty acids are associated with chronic diseases
- Describe how dietary requirements for essential fatty acids can be determined.

Introduction

Essential polyunsaturated fatty acids, of both the n-3 and n-6 series, play a critical role in a myriad of complex biologic and metabolic pathways. They thereby affect various processes relevant to human health and disease throughout the life course, from fetal and infantile neurodevelopment to immune modulation, with an impact on diverse conditions as asthma, rheumatoid arthritis, inflammatory bowel disease, and atherosclerosis.

This review focuses on basic science research and clinical/epidemiological studies relating to essential polyunsaturated fatty acids, with an emphasis on areas of importance to developing countries, specifically those pertaining to mother and child health. Attempts to assess dietary requirements of essential fatty acids and maintain their adequate intake during pregnancy, lactation, and early childhood, in vulnerable middle- and low-income populations, will be addressed.

E. Granot (✉)
Department of Pediatrics, Kaplan Medical Center, Rehovot,
Hebrew University—Hadassah Medical School, Jerusalem, Israel
e-mail: ettie.granot@ekmd.huji.ac.il

R.J. Deckelbaum
Institute of Human Nutrition, Departments of Pediatrics and Epidemiology,
Columbia University Medical Center, New York, USA
e-mail: rjd20@cumc.columbia.edu

S. de Pee et al. (eds.), *Nutrition and Health in a Developing World*,
Nutrition and Health, DOI 10.1007/978-3-319-43739-2_14

Essential Fatty Acids and Their Metabolism

Essential polyunsaturated fatty acids (PUFA) cannot be synthesized in the body and must be ingested in food. There are two series of essential fatty acids: omega 3 fatty acids characterized by a double bond at the third carbon from the methyl end of the fatty acid and omega 6 fatty acids which have a double bond at the position of the sixth carbon from the methyl end.

The n-3 fatty acids originate from synthesis in plants and algae. The main n-3 fatty acid consumed is alpha-linolenic acid (ALA)(C 18:3 n-3). The major dietary source of two n-3 fatty acids, eicosapentanoic (EPA) acid and docosahexaenoic acid (DHA), is fish. The proportion of linolenic acid that can be converted to the longer chain n-3 fatty acids EPA (C 20:5 n-3) and DHA (C 22:6 n-3) is very low, estimated at less than 1% [1–4].

The main essential n-6 fatty acid present in diet, from which longer n-6 fatty acids are synthesized, is linoleic acid (LA) (C 18:2 n-6). The longer chain-6 fatty acid arachidonic acid (AA) (20:4,n-6), is also present in diet, but to a lesser degree. Linoleic acid can be metabolized to arachidonic acid (C 20:4 n-6). Arachidonic acid is further metabolized, by chain elongation, via docosatetraenoic acid (C 22:4 n-6) to tetracosapentaenoic acid (C 24:5 n-6). The metabolism of n-3 and n-6 fatty acids is interrelated and uses the same enzymes for elongation and desaturation; high intakes of the n-6 linoleic acid inhibit the metabolism of the n-3 linolenic acid so that the conversion of linolenic acid to DHA is decreased (Fig. 14.1).

In 1999 an NIH sponsored Workshop on the Essentiality of the Recommended Dietary Intakes for n-6 and n-3 Fatty acids [5] emphasized the importance of decreasing n-6 PUFA in face of an increase in dietary n-3 PUFAs, in order to reduce adverse effects of excesses of AA and its eicosanoid products

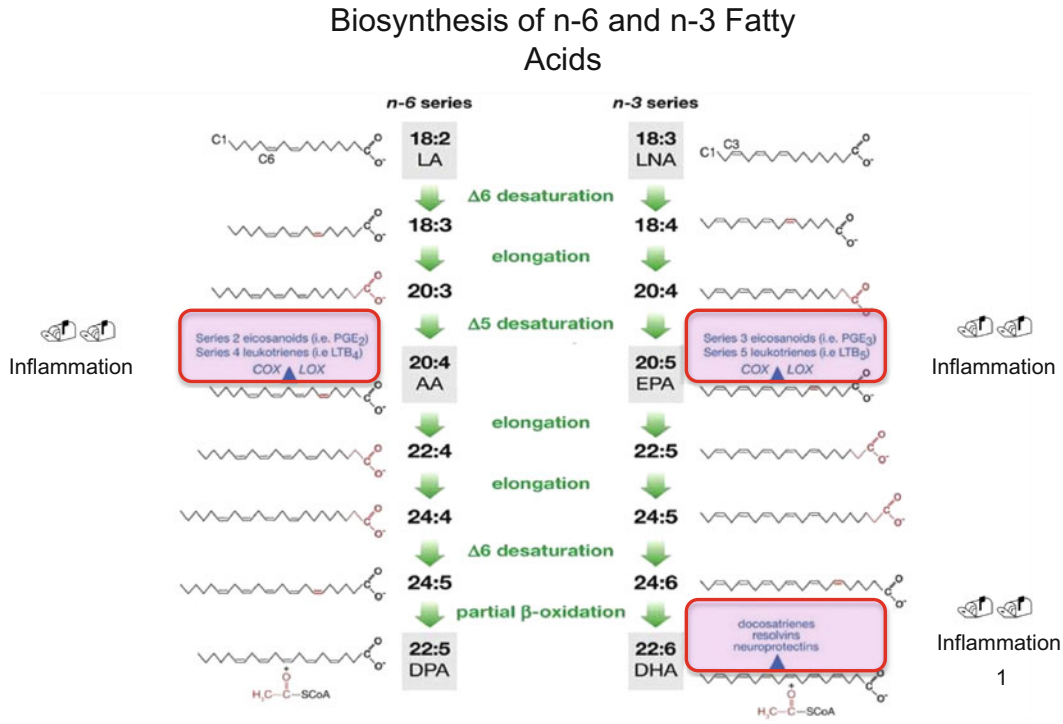

Fig. 14.1 Schematic illustration of major n-3 and n-6 fatty acids derived from linoleic acid and alpha-linolenic acid. Adapted from Marszalek and Lodish [99]

that can occur when too much linoleic acid and AA are present in the diet. Linoleic acid converts to AA via the enzyme Δ-6 desaturase, which is the same enzyme necessary to desaturate alpha-linolenic acid, the parent compound of the n-3 class.

Traditionally human diets contained almost equal amounts (1–2:1) of n-6 and n-3 fatty acids. Over the past 100–150 years there has been a decrease in n-3 FA intakes and an enormous increase in consumption of n-6 FA, due to intake of vegetable oils from corn, sunflower seeds, safflower seeds, and soybeans. In Western diets the n-6/n-3 ratio is estimated at 15:1–16:1 but can reach as high as 20–30:1 [6–8].

Reducing the large amounts of dietary n-6 LA (present in corn, safflower and soybean oils) or increasing dietary ALA will better balance n-6/n-3 ratios. The known competition between the two pathways of essential fatty acids is very difficult to untangle biologically and it is not known in what proportions C20 & C22 unsaturated long-chain fatty acids will be produced from different dietary mixtures of linoleic and alpha-linolenic acids.

Polyunsaturated Fatty Acids and Immunomodulation

Polyunsaturated fatty acids are basic constituents of phospholipid membranes and affect membrane structure and fluidity. They are also precursors of eicosanoids (prostaglandins, prostacyclins, thromboxanes, and leukotrienes) via which they exert an effect on the immune response. PUFA also modulate nuclear receptors and intracellular enzyme activities. Leukotrienes derived from AA via the lipoxygenase pathway stimulate production of proinflammatory cytokines, and the prostanoids derived from AA via the cyclooxygenase pathway promote vasoconstriction and aggregation of thrombocytes. EPA competes for the same initial enzymes in the lipoxygenase and cyclooxygenase pathways leading to inhibition of eicosanoid synthesis from AA. Thus, n-6 FA are expected to shift the physiologic state to one that is proinflammatory and prothrombotic while n-3 FA are expected to result in production of eicosanoids that posses more anti-inflammatory, antithrombotic, and vasodilation properties [9].

PUFA are incorporated into membrane phospholipids, specifically in distinct micro domains of the plasma membrane which are known as lipid rafts. Lipid rafts play a critical role in signal transduction; PUFA regulate gene expression either directly by interaction with nuclear receptors as the peroxisome proliferators-activated receptor (PPAR) and liver X receptor (LXR), or indirectly via pathways initiated at the plasma membrane such as T cell receptors of Toll—like receptors. The effects of PUFAs on various nuclear receptors are receptor-specific and fatty-acid-specific. PUFAs which activate PPARγ inhibit expression of proinflammatory cytokines. Conversely, as LXR agonists exert an anti-inflammatory effect via inhibition of COX2 and IL6, PUFAs that inhibit the LXR, would be expected to have a proinflammatory effect [10, 11]. n-3 fatty acids, in contrast to saturated fatty acids, have been shown to decrease endothelial lipase which is one of the lipases synthesized and secreted by macrophages [12]. In mice, endothelial lipase deficiency is associated with a decrease in atherosclerotic lesions. PPARγ, highly expressed in macrophages—derived foam cells in atherosclerotic plaques is regulated by EPA, leading to a decrease in endothelial lipase and a parallel decrease in proinflammatory markers and increase in anti-inflammatory markers [12].

Recently, novel di- and trihydroxy-containing n-3 fatty acid-derived mediators have been defined. These mediators named resolvins and protectins are of importance in terminating a state of inflammation. The noninflamed state is described not as a passive process but rather as an actively regulated program of resolution [13, 14].

Associations Between N-3 and N-6 PUFA Dietary Intakes and Disease

Various epidemiological studies link a high dietary n-6/n-3 ratio with an increased risk of cardiovascular diseases, diabetes, asthma, autoimmune disorders, and even cancer suggesting that a Western-type diet based on a high dietary intake of n-6 FA can have detrimental effects [8]. Adopting a Western diet has been shown to be associated with increased morbidity from noncommunicable diseases—some examples are reviewed below.

Noncommunicable Diseases

Raheja et al. [15] reported a sharp increase in the prevalence of non-insulin-dependent diabetes mellitus and coronary artery disease among the upper socioeconomic classes in India after they adopted diets high in total fat and with high n-6/n-3 ratios. Indeed, in Japanese women a low incidence of breast cancer has been observed among those women who conserved their traditional diet which contains fats derived mainly from marine sources rich in n-3 PUFA. Similarly, Greenland Inuits have a very low incidence of breast cancer, cardiovascular diseases, and autoimmune disorders despite their relatively high fat diet, presumably because of its high n-3 FA content [8, 16, 17].

While results are mixed, administering of n-3 PUFA as fish oil or as individual or a combination of n-3 FA, in different ratios and amounts, has been studied in the prevention and control of a multitude of disease states including CHD, hypertension, type II diabetes, rheumatoid arthritis, renal disease, inflammatory bowel disease, asthma, multiple sclerosis, depression, schizophrenia, and Alzheimer's dementia [18, 19].

Increased dietary amounts of n-3 FA have proven beneficial in the secondary prevention of cardiovascular disease; a ratio of 4:1 was associated with a 70% decrease in total mortality [20, 21]. Yet, despite other studies in cardiovascular patients demonstrating beneficial effects of n-3 FA supplementation [22], a systematic review of 48 RCT and 26 cohort studies reached the conclusion that the positive effects of n-3 FA supplementation is nonsignificant [23]. Indeed, more recent, large intervention studies with marine n-3 fatty acids failed to replicate earlier findings of reduced cardiovascular mortality in at-risk patients [24, 25]. Attempts to reconcile these conflicting data emphasize different background intakes of the different n-6 and n-3 fatty acids in the different populations studied, sample size differences, timing of initiation of the n-3 fatty acid supplements, and the relative amounts of EPA versus DHA used [26].

Asthma

Population studies in Australia showed that regular fish intake was associated with a 50% reduction in asthma prevalence and increased airway responsiveness [27] and that children whose parents reported eating oily fish have significantly less asthma [28]. Yet, the effect of n-3 FA is likely complex—in patients with asthma, an n-6/n-3 ratio of 5:1 was linked to a beneficial effect whereas a 10:1 ratio had adverse consequences [29]. In a Japanese study of 6–15-year olds, which compared 1673 asthmatics versus 22,109 healthy children, increased fish intake (1–2/week) was associated with a significantly higher prevalence of asthma [30]. However, a review of 9 RCT (in both children and adults) comparing n-3 FA supplementation for at least 3 weeks versus placebo found no convincing evidence regarding the ability of n-3 FA to improve asthma symptoms [31]. These differences might be related to different baseline n-3 (or n-6) intakes in different populations.

Other Conditions

Studies on the effect of n-3 FA on ADHD, autism, and related disorders similarly yielded conflicting results [32, 33] but given their relative safety and general health benefits n-3 FA are considered as a promising complementary approach to standard treatments [34].

Similarly, when assessing benefit of n-3 PUFA supplementation in rheumatoid arthritis patients (on pain, swollen joints, laboratory parameters of disease activity), in over 19 RCTs, results are inconsistent, but in some studies patients did show a definite improvement in clinical findings and laboratory parameters [35, 36].

In cancer patients there is limited data regarding benefits of n-3 FA. A dietary ratio of n-6/n-3 of 2.5:1 reduced rectal cell proliferation in patients with colorectal cancer but interestingly, no beneficial effect was observed with the same amount of n-3 but when the ratio of n-6/n-3 was maintained at 4:1 [37, 38]. A recent review highlighting controversies relating to dietary fatty acids in disease [39] addressed the contradictory studies relating to the effect of fish oil and fish oil fatty acids on lung cancer and the conflicting data on the association between plasma phospholipids fatty acids and prostate cancer [40, 41]. An association has been reported between higher blood n-3 fatty acids and increased cancer prostate cancer risk [42, 43]. Yet, a meta-analysis of eight prospective studies concluded that intake of marine n-3 fatty acids is not associated with prostate cancer development [44]. Currently, there is insufficient evidence for establishing any relationship of PUFA consumption with cancer.

It is noteworthy that results of different trials are not consistent, likely as amount and type of PUFA used are not uniform and include different dietary fish, fish oils and/or flaxseed oil, rapeseed oil, primrose oil and EPA, DHA and ALA in variable doses and ratios and for different time periods. As the conversion of ALA to EPA and DHA is highly inefficient, the beneficial effect of rapeseed or flaxseed oil may result from the ALA itself. The potential, independent immunomodulatory effects, if any, of ALA are yet to be determined [45]. Furthermore, optimal doses or combinations of n-3 fatty acids, or perhaps optimal n-6/n-3 ratios or absolute amounts of n-6 FA and n-3 FA, may be disease dependent so that in future treatment should be tailored to the specific disease/condition and even to its degree of severity.

Effect of N-6 and N-3 Fatty Acids in Pregnancy, Lactation, and Infancy

DHA is deposited in appreciable amounts in the central nervous system during the perinatal brain growth spurt with fetal DHA accretion during the third trimester of gestation estimated at 45–60 mg/day. It is noteworthy that AA accretion occurs mainly postnatally.

Dietary fat intakes and specifically intakes of AA and DHA are important during pregnancy and early infancy, affecting pregnancy outcome, fetal growth, and infants' neural maturation and retinal function [46–50]. In a study encompassing 11,875 pregnant women, low maternal sea food consumption was found to be associated with offspring achieving lower social scores, decreased fine motor skills, and higher risk of IQ scores at the lowest quartiles [51]. Observation and intervention studies involving pregnant and lactating women and infants-fed DHA-supplemented formulas show that a greater intake of DHA is associated with better scores on tests of visual and neural development in infancy and early childhood [4, 52].

Although the fractional conversion of ALA to n-3 long-chain PUFAs may be greater in women than in men, it is limited and cannot meet the increased demands for DHA which must therefore be met by an increase in dietary DHA intake. The recommended daily allowance of DHA during pregnancy and lactation is 300 mg/day [5] with women urged to aim at an intake of at least

200 mg/day [53]. This desired intake can be reached with the consumption of one to two portions of fish per week, of which tuna, herring, mackerel, salmon, and trout are those richer in EPA and DHA. As for AA, plasma and tissue content is relatively stable and not influenced by the dietary intake of preformed AA. There is no indication that women of childbearing age with an adequate dietary intake of the precursor fatty acid, linoleic acid, need an additional supply of AA [53]. Most pregnant women do not meet the recommended dietary intake of DHA. In a survey of pregnant Canadian women mean DHA intake was only 82 ± 33 mg/day and in 90% of low-income Midwest American women intake was far below 300 mg/day [54, 55].

Levels of maternal DHA decline with multiple pregnancies; levels have been shown to be significantly less in multiparous compared with primiparous mothers and when pregnancies are closely spaced [56]. Low consumption of fish has been linked to a higher percentage of preterm deliveries [57, 58] and the effects of n-3 long-chain PUFA supplementation (150–1200 mg of DHA daily or up to 2.7 gr total n-3 long-chain PUFA), in pregnant women, on length of gestation and birth weight have been evaluated. In a number of trials a slight increase in birth weight, and length of gestation coupled with a reduced risk of preterm delivery, were observed [59–61].

One of us (E.G) recently studied the effect of 400 mg/day of DHA supplementation starting after the 12th week of gestation in multiparous mothers with all of them in at least their third pregnancy (40% in their fifth–seventh pregnancies). Gestational length and infants' birth weight, in the DHA-supplemented group were compared to a non-supplemented control group and were also compared within groups to the mothers' previous pregnancies. DHA supplementation did not have any effect on the infants' birth weight. However, an effect of DHA supplementation on length of gestation was observed in highly multiparous mothers, those with six or more pregnancies [62].

Long-Chain Polyunsaturated Fatty Acids in Human Milk

Maternal dietary intake of DHA affects levels of DHA in breast milk. In a study comparing the composition of long-chain PUFAs in diet and breast milk of Chinese women from a rural area and Swedish women residing in Stockholm the Chinese diet was found to contain more linoleic acid and less AA, EPA, and DHA than that of Swedish mothers [63]. These dietary differences were mirrored in breast milk fatty acid composition; the ratio of AA to DHA was 3.1 in the Chinese women's breast milk and 1.6 in that of Swedish mothers [63]. A comparison of breast milk fatty acid composition of Chinese women residing in five different geographical regions demonstrated a highly variable AA to DHA ratio (g/g) with mean levels of 2.77 in inland areas to 0.42 in coastal/sea areas [64]. In a meta-analysis that considered 65 studies encompassing 2474 women worldwide, breast milk fatty acid composition demonstrated mean DHA concentrations (% of total fatty acids) of 0.32 ± 0.22 (range 0.06–1.4%) and mean AA concentrations of 0.47 ± 0.13(% of total fatty acids) (range 0.24–1.0%). The mean ratio of AA to DHA in breast milk, even within the same geographical region, varies widely. The highest breast milk DHA concentrations were observed primarily in coastal populations and were associated with marine food consumption [65]. Populations with the highest breast milk DHA concentrations, in the range of 0.6–1.4%, included women from Japan, Philippines, Congo, Sweden, Dominican Republic, and Canadian women residing in arctic regions. All of these, with the exception of Congo, are coastal or island populations. In Congo, proximity to lakes and rivers likely results in high dietary fish intake. The lowest DHA levels were noted in breast milk samples from Pakistan, rural South Africa, Canada, the Netherlands, and France with DHA levels in the range of 0.06–0.14% [65]. These populations reside inland or are from developed countries, both of which are usually associated with low marine food consumption. Indeed, in Chinese women residing in the coastal regions of China breast milk mean DHA levels are 2.78% of total fatty acids whereas levels were only 0.68% in breast milk of Chinese women residing in inland rural areas of

China [64]. Notably, the concentration of AA in breast milk samples is much less variable than that of DHA. Based on these studies, the best estimates of worldwide mean breast milk AA and DHA concentrations (% of total fatty acids) are 0.47 for AA and 0.32 for DHA [65].

The amount of AA and DHA in infant formulas is currently based on these mean breast milk levels, with a ratio of AA/DHA of 1–1.5:1, thereby aiming to have infant formulas which simulate the essential fatty acid composition of human milk. The recommended DHA level in infant formulas is 0.2–0.5% of fatty acids with the amount of AA being at least equal to the DHA level. The total fat content in most infant formulas is 4.4–6.0 g/100 kcal, equivalent to ∼40–54% of energy content.

The comparison of levels of AA and DHA in mature and premature human milk is of special interest as both play a pivotal role in brain development and visual function maturation. The critical period of placental transfer of these fatty acids is believed to be in the third trimester of pregnancy. It has been estimated that approximately 45–60 mg of DHA per day accrues in the fetal brain during this time period [47, 49]. Thus, in infants born prematurely depleted DHA stores, coupled with limited ability to synthesize long-chain PUFAs [4] and enhanced needs due to accelerated growth, places them at high risk for ARA and DHA deficiency.

Long-chain PUFAs and especially DHA are preferentially transported across the placenta to the developing fetus and accumulate extensively in the fetal brain during the last trimester of pregnancy. Thus, infants born prematurely are at a disadvantage as their body stores of long-chain PUFAs are limited while at the same time requirements for fatty acid deposition in their rapidly growing tissues are high [66]. After delivery, endogenous long-chain PUFAs synthesis is relatively low and supply of long-chain PUFAs to the breast-fed infant depends on the amount of these fatty acids in breast milk.

Human milk fatty acid composition is influenced by factors such as maternal parity, maternal diet, and stage of lactation. As infants born prematurely have increased requirements for long-chain PUFAs, it is of interest to determine whether human milk fatty acid composition is also influenced by duration of pregnancy. To date, studies on long-chain PUFAs content in milk of mothers who gave birth to preterm infants have yielded conflicting results [67–70].

One of us (E.G) recently compared fatty acid composition in human milk of mothers giving birth to full-term and preterm infants. In the mothers of preterm infants, breast milk fatty acid composition was also studied during the first 2 weeks after delivery [71]. This study did not observe differences in the proportion of either AA or DHA nor did these levels increase, in breast milk of mothers giving birth to preterm infants during the 2 weeks post delivery period studied. Furthermore, even in the "very small" premature infants (26–30 weeks gestation), included in our study, mothers' breast milk AA and DHA levels did not differ from mean levels in breast milk of mothers giving birth to full-term infants. Thus, the results of this study are in accord with the studies by Luukkainen [68] and Kovacs [70] showing that breast milk of mothers of preterm infants does not compensate for the special needs and increased requirements for long-chain PUFAs, which result from prematurity. Optimal daily amount of AA & DHA required by the premature infant should be defined so one can determine if there is a need to add essential fatty acids not only to infant formulas but also to supplement breastfeeding mothers.

Breastfeeding confers protection against infections during infancy. Breast-fed infants have an enhanced local humoral immune response, resulting in a lower prevalence of gastrointestinal and respiratory tract infections than in formula—fed infants [72, 73]. Exclusive breastfeeding for the first few months has also been suggested to be protective against the development of atopic disease [74]. Immunoglobulins, lymphocytes, proteins like lactoferrin and lysozyme, which are present in breast milk, play a specific immunologic role.

Another component, which may be of high importance to maturation and function of the immune system, is the fatty acid pattern of dietary milks. Human milk contains long-chain PUFAs (20–22 carbons) of both n-3 and n-6 class which constitute ∼2% of total fatty acids and which are undetectable in unsupplemented formulas prepared from vegetable oils. The type of dietary milk which infants consume results in changes in the fatty acid pattern of cell membrane phospholipids [75].

As the ratio of arachidonic acid-derived eicosanoids and those derived from n-3 fatty acids has been suggested to play a role in immune modulation [76, 77] we designed a study to assess whether the beneficial effects of breast milk on the immune response might be related to its essential fatty acid composition. We measured RBC membrane fatty acid composition, as a surrogate marker for WBC membrane fatty acid composition, by gas–liquid chromatography in breast-fed and non-supplemented formula-fed, 2–4 month old, infants. Release of the proinflammatory cytokines IL-1 and TNF was measured in whole blood culture in bacterial endotoxin stimulated and nonstimulated cells. Significant differences were observed in cell membrane fatty acid composition between breast-fed and formula-fed infants; breast-fed infants had a significantly greater percentage of n-3 fatty acids and specifically a twofold greater level of DHA than infants fed a non-supplemented formula, with similar membrane levels of AA and % total n-6 fatty acids. Despite differences in cell membrane fatty acid composition, the release of proinflammatory cytokines by immunocompetent cells did not differ between breast-fed and formula-fed infants [78]. Although our study failed to prove our hypothesis that increased mononuclear cell membrane DHA levels would likely reduce cellular production of proinflammatory cytokines, it was credited for providing "a new glimpse into neonatal nutrition and its effects on the immune response" [79].

Various parameters relating to the immune response have been compared in infants-fed human milk, infants-fed non-supplemented formulas, and infants-fed long-chain PUFAs-supplemented formulas. Infants receiving long-chain PUFAs fortified formula showed an increase in the proportion of activated "memory" CD4 and CD8 cells [80], but no changes were observed in older children receiving AA and DHA for a 7-month period [81].

In vitro and in vivo studies in animals and humans have demonstrated that long-chain PUFAs reduce release of the proinflammatory cytokines IL-2 TNFα and IFNγ from mononuclear cells [82–84], although other researchers did not observe any change in IFNγ release following long-chain PUFAs supplementation [81].

As maternal DHA levels decline with multiple, closely spaced pregnancies we questioned whether DHA supplementation during pregnancy and lactation, in a population with a high percentage of multiparous mothers would affect the immune response of their infants, solely breast-fed up to the age of 4 months [85]. DHA supplementation did not exert an effect on the humoral immune response and infants in both groups did not differ in levels of immunoglobulins of IgG, IgM, and IgA classes and the specific antibody response as reflected by measuring the titer of antibodies to HBsAg after two doses of vaccine (at 2 days and at 1 month of age) was similar in both groups.

Infants in the DHA-supplemented group had a similar number of CD4 cells as the non-supplemented group but the number of CD8 cells was significantly reduced (CD4/CD8 ratio 2.6 in DHA-supplemented infants as compared with 1.9 in the non-supplemented group). Of CD4 cells the fraction of CD45RA+ cells, representing naïve helper cells, was significantly higher in the DHA-supplemented group and the proportion of CD8 + CD45RO + activated cells was significantly higher, constituting 35% of CD8 cells as compared with 16% in the non-supplemented group. In both CD4 & CD8 cells the production of IFNγ was found to be markedly lower in lymphocytes of the infants in the DHA-supplemented group [85].

Thus, in our study DHA supplementation during pregnancy and lactation did not result in changes in the infants' humoral immune response, as assessed by levels of antiHBs antibodies and total immunoglobulins, but did affect lymphocyte subset profile and cytokine production. A lymphocyte profile with a higher percentage of CD4 naïve cells and decreased IFNγ production in both CD4 & CD8 cells is likely compatible with attenuation of a proinflammatory response. The clinical implications of the changes observed in cellular immune response are as yet speculative and whether supplementation of DHA to breast feeding mothers confers an advantage to their infants remains to be elucidated.

Essential Fatty Acid Requirements in Healthy Infants, Children, and Adults

The Joint FAO/WHO Expert Consultation on Fats and Fatty Acids, Geneva 2008, recommended that total n-3 FA intake range between 0.5 and 2.0% of total energy with a minimum dietary requirement of alpha linoleic acid being 0.5% energy [86]. For adult males and nonpregnant, nonlactating females 0.25–0.5 g/day of EPA plus DHA, together, is recommended, with insufficient evidence to set a specific minimum intake of either EPA or DHA alone; both should be consumed. For pregnant and lactating mothers the minimum intake, for both their own optimal health and that of the fetus and infant, is 0.3 g/day of EPA&DHA of which at least 0.2 g/day should be DHA [86]. As for n-6 FA there is no sufficient data for establishing a precise quantitative estimate of the LA requirement needed in order to prevent deficiency. An adequate intake of LA of 2–3% of energy is proposed. Arachidonic acid is not essential for a healthy adult whose habitual diet provides LA at an amount that is at least 2.5% of total caloric energy supply. There is as yet no compelling scientific evidence upon which to base recommendations regarding specific ratios of dietary n-6 FA to n-3 FA or ratio of LA to ALAs [86, 87].

The expert panel reiterated the recommendations that the minimum concentration of DHA in infant formulas and baby foods should be 0.2% of total fatty acids and should not exceed 0.5% of total FA. Levels of added AA should, at least, equal that of added DHA. The amount of EPA added should not exceed the amount of DHA. For infants 0–6 months of age AA should be supplied in the diet within the range of 0.2–0.3% E, based on human milk composition. For older infants (6–24 months of age), the LA adequate intake range recommended is 3.0–4.5% of total caloric energy intake. Sources of long-chain PUFAs during the first year of life include breast milk, infant formulas, or follow-on formulas enriched with long-chain PUFAs and complementary foods such as eggs and fatty fish. Highly refined oils from single-cell organisms (algae and fungi), eggs, or fish are appropriate as DHA and AA sources for use in infant formulas and complementary foods [86].

Essential Fatty Acids and Specific Needs in Developing Countries

A special concern is the lack of data regarding dietary intake of PUFA in low and middle income countries (LMICs). A recent review compiled information, from the few studies available, on the content of PUFA in breast milk and in major foods using national food balance sheets from the United Nations' Food and Agriculture Organization Statistical Database (FOASTAT) for 13 LMICs [88]. This review indicated that breast milk DHA content is very low in populations living mainly on a plant-based diet but is higher in fish-eating communities. Per capita supply of fat and n-3 FA increases markedly with increasing gross domestic product (GDP). In most of the 13 countries surveyed, 70–80% of the supply of PUFA comes from cereals and vegetable oils, some of which have very low alpha-linolenic acid content. In the nine countries with the lowest GDP, the total n-3 FA supply was found to be below or close to the lower end of the recommended intake range (0.4% of energy supply) for infants and children and below the minimum recommended level (0.5% of energy) for pregnant and lactating women [88]. As breast milk is one of the best sources of alpha-linolenic acid and DHA, breast-fed infants are less likely to be at risk of insufficient intakes than infants who are not breast-fed. If needed, where maternal diets have insufficient n-3 intakes mothers can be given DHA supplementation during pregnancy and lactation; this may be particularly of benefit when breast feeding is continued for 2 years or more [89].

The issue of optimal infant nutrition, specifically of n-3 FA "fortified" foods is an area of interest, with a special emphasis on the needs of infants and young children from low-income populations,

with a high prevalence of malnutrition. The potential for small quantity lipid-based nutrient supplements (LNS), i.e., 20 g/d providing <120 kcal/d to promote growth and development after 6 months of age, is currently being investigated. A recent study showed that infants receiving such supplements do not reduce their intake of breast milk so that the beneficial effects of breast milk are not concomitantly decreased [90, 91]. In infants who are 6 months of age, the introduction of complementary feeding coupled with continued breast feeding is of special interest. Ensuring an adequate intake of micronutrients, (including calcium, iron, zinc, B vitamins), fat-soluble vitamins (vitamins A, D, E, and K), appropriate amounts of protein and fat, and specifically the recommended allowance of EFA, is of major importance [92].

The Food and Agriculture Organization (FAO) reports that adequate intakes of ALA should be 0.4–0.6% of energy intake and that of LA 3.0–4.5% of energy intake, for children aged 6–23 months. For children residing in LMICs, where the prevalence of underweight is high, essential fatty acid recommendations based on percentage of daily energy intake would be expected to result in calculated lower recommended daily amounts of EFA than those of children in well-nourished populations. Furthermore, the lower stores of EFA that these children likely have and the conversion of EFA to longer chain derivatives, which is thought to be defective in malnourished children, should also be considered [94–96]. Daily recommended intakes for EFA in LMICs should be adjusted accordingly and not be based only on the children's body weight as this would likely underestimate their needs and increase the risk of inadequate intake and consequent nutritional deficiency. Yang and Huffman [93] suggested that adequate requirements should therefore be calculated based on the WHO Reference Growth Standards, of median body weights of children of similar age, thereby "correcting" the lower estimated EFA in undernourished children.

Enhancing intake of alpha-linolenic acid through complementary plant food products (soy beans, soy oil, canola oil, lipid-based supplements) is feasible but due to the low conversion rates of ALA to DHA it may be more efficient to enhance DHA status by increasing fish consumption or DHA fortification. An industry that specializes in farmed fish that are fed specific oils, and in the growing of microalgae, on a large scale, can serve as adequate sources of EPA and DHA. Obtaining n-3 fatty acids through genetically selected or modified plants is also being studied [97].

Fortification of foods with n-3 long-chain PUFA, specifically DHA, is of growing interest. Designing products with these long-chain fatty acids for developing countries is especially challenging as they should be designed so as to address the nutritional needs of the target population and to withstand environmental conditions that may affect the fatty acids' stability and cause them to undergo lipid peroxidation. Other concerns include palatability of fortified foods, and preparation of appropriate formulations (powder, capsules, oils) according to the age group (infants, adults) for which they are intended [98].

In order to achieve adequate intake of EFA worldwide recognition of their importance is needed. Effective strategies to address adequate intakes should address the optimal balance of dietary LA and ALA based on the metabolism of these fatty acids, determine the appropriate recommended intake for different periods of life (pregnancy, infancy, early childhood), and allow for differences in requirements based on nutritional status. Still, more information is needed to recommend appropriate EFA intake in populations with undernutrition.

Conclusion

Long-chain polyunsaturated fatty acids act as major mediators of processes relating to health and disease throughout the life course both in developed and LMIC populations. Their role in fetal and early infancy is critical and their intake has also been linked to major causes of morbidity and mortality as cardiovascular diseases, diabetes, asthma, autoimmune disorders, and even cancer.

The optimal intake of essential fatty acids at various milestones in life and whether intake should be modified to account for the differing needs in LMICs and in less economically privileged populations warrants further research.

Discussion Points

- What are the key mechanisms through which essential fatty acids affect health throughout the lifespan?
- Describe the factors that affect the dietary requirements for essential fatty acids.
- How can changing dietary patterns in the world affect the essential fatty acid status of people living in low and middle income countries?
- What role can essential fatty acids have as part of nutritional interventions in low and middle income countries?

References

1. Salem N Jr, Wegher B, Mena P, Uauy R. Arachidonic and docosahexanoic acids are biosynthesized from their 18-carbon chain precursors in human infants. Proc Natl Acad Sci USA. 1996;93:49–54.
2. Hussein N, Ah-Sing E, Wilkinson P, et al. Long-chain conversion of ^{13}C linoleic acid and alpha linolenic acid in response to marked changes in their dietary intake in men. J Lipid Res. 2005;46:269–80.
3. Goyens PL, Spilker ME, Zock PL, et al. Compartmental modeling to quantify alpha linolenic acid conversion after longer term intake of multiple tracer boluses. J Lipid Res. 2005;46:1474–83.
4. Innis SM. Omega3 fatty acids and neural development to 2 years of age: do we know enough for dietary recommendations? J Pediatr Gastroenterol Nutr. 2009;48:S16–24.
5. Simopoulos AP, Leaf A, Salem N Jr. Workshop Statement on the essentiality of and recommended dietary intakes for omega-6 and omega-3 fatty acids. Prostagland Leuk Essent Fat Acid. 2000;63:119–21.
6. Simopoulos AP. Essential fatty acids in health and chronic diseases. Am J Clin Nutr. 1999;70:560S–9S.
7. Simopoulos AP. The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed Pharmacother. 2002;56:365–79.
8. Yam D, Eliraz A, Berry EM. Diet and disease—the Israeli paradox: possible dangers of a high omega 6 polyunsaturated fatty acid diet. Isr J Med Sci. 1996;32:1134–43.
9. Seo T, Blaner WS, Deckelbaum RJ. Omega 3 fatty acids: molecular approaches to optimal biological outcomes. Curr Opin Lipidol. 2005;16:11–8.
10. Fritsche K. Fatty acids as modulators of the immune response. Ann Rev Nutr. 2006;26:45–73.
11. Calder PC. Polyunsaturated fatty acids and inflammatory processes: new twists in an old tale. Biochimie 2009; 791–795.
12. Jung UJ, Torrejon C, Chang CL, Hamai H, Worgall TS, Deckelbaum RJ. Fatty acids regulate endothelial lipase and inflammatory markers in macrophages and in mouse aorta, a role for PPARγ. Arterioscler Thromb Vasc Biol. 2012;32:2929–37.
13. Weylandt KH, Chui CY, Gomolka B, Waechter SF, Wiedenmann B. Omega-3 fatty acids and their lipid mediators: towards an understanding of resolving and protectin formation. Prostaglandins Other Lipid Mediat. 2012; 97:73–82.
14. Bannenberg GL, Chiang N, Ariel A, Arita M, Tjonahen E, Gotlinger KH, Hong S, Serhan CN. Molecular circuits of resolution: formation and actions of resolvins and protectins. J Immunol. 2005;174:4345–55.
15. Raheja BS, Sadikot SM, Phatak RB, Rao MB. Significance of the w-6/w-3 ratio for insulin action in diabetes. Ann NY Acad Sci. 1993;683:258–71.
16. Horrobin DF, Huang YS. The role of linoleic acid and its metabolites in the lowering of plasma cholesterol and the prevalence of cardiovascular disease. Int J Cardiol. 1987;17:241–55.
17. Kromann N, Green A. Epidemiological studies in the Upernavik district, Greenland. Incidence of some chronic diseases 1950–1974. Acta Med Scand. 1980;208:401–6.

18. Lorente–Cebrian S, Costa AG, Navas–Carretero S, Zabala M, Martinez JA, Moreno-Aliaga MJ. Role of omega-3 fatty acids in obesity, metabolic syndrome and cardiovascular disease: a review of the evidence. J Physiol Biochem 2013; 69:633–651.
19. Cabre E, Manosa M, Gassull MA. Omega-3 fatty acids and inflammatory bowel disease—a systematic review. Br J Nutr. 2012;107(Suppl 2):S240–52.
20. de Lorgenile M, Renaud S, Mamelle N, Salen P, Martin JL, Monjaud I, Guidollet J, Touboul P, Delaye J. Mediterranean alpha linolenic acid-rich diet in secondary prevention of coronary artery disease. Lancet. 1994;343:1454–9.
21. Saravanan P, Davidson NC, Schmidt EB, Calder PC. Cardiovascular effects of marine omega-3 fatty acids. Lancet. 2010;376:540–50.
22. Wang C, Harris WS, Chung M, et al. w-3 fatty acids from fish or fish-oil supplements but not alpha-linolenic acid acid, benefit cardiovascular disease outcomes in primary and secondary prevention studies: a systematic review. Amer J Clin Nutr. 2006;84:5–17.
23. Hooper L, Thompson RL, Harrison RA, Summerbell CD, Ness AR, Moore HJ, Worthington HV, Durrington PN, Higgins JP, Capps NE, Riemersma RA, Ebrahim SB, Smith G. Risks and benefits of omega 3 fats for mortality, cardiovascular disease and cancer: a review. BMJ. 2006;332:752–60.
24. Kromhout D, Giltay EJ, Geleijnse JM. For the Alpha Omega Trial Group. W-3 fatty acids and cardiovascular events after myocardial infarction. N Engl J Med. 2010;363:2015–26.
25. Rauche B, Schiele R. Schneider S et al OMEGA, a randomized placebo controlled trial to test the effect of highly purified omega-3 fatty acids on top of modern guideline adjusted therapy after myocardial infarction. Circulation. 2010;122:2152–9.
26. Deckelbaum RJ, Calder PC. Different outcomes for omega -3 heart trials: why? Curr Opin Clin Nutr Metab Care. 2012;15:97–8.
27. Peat JK, Salome CM, Wollcock AJ. Factors associated with bronchial hyper responsiveness in Australian adults and children. Eur Respir J. 1992;5:921–9.
28. Hodge L, Salome CM, Peat JK, Haby MM, Xuan W, Woolcock AJ. Consumption of oily fish and childhood asthma risk. Med J Aust. 1996;164:137–40.
29. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced asthma symptoms with w3fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr. 1997;65:1011–7.
30. Takemura Y, Sakurai Y, Honjo S, Tokimatsu A, Gibo M, Hara T, Kusakari A, Kugai N. The relationship between fish intake and the prevalence of asthma. Prev Med. 2002;34:221–5.
31. Woods RK, Thien FC, Abramson MJ. Dietary marine fatty acid (fish oil) for asthma in adults and children. Cochrane Database Syst Rev 2002: CD001283.
32. Richardson AJ, Montgomery P. The Oxford-Durham study: a randomized, controlled trial of dietary supplementation with fatty acids in children with developmental coordination disorder. Pediatrics. 2005;115:1360–6.
33. Amminger GP, Berger GE, Schafer MR, Klier C, Friedrich MH, Feucht M. Omega-3 fatty acids supplementation in children with autism: a double blind, randomized, placebo-controlled pilot study. Biol Psychiatr. 2007;61:551–3.
34. Richardson AJ. Omega-3 fatty acids in ADHD and related neurodevelopmental disorders. Int Rev Psychiatry. 2006;18:155–72.
35. Kremer JM. Omega-3 fatty acid supplements in rheumatoid arthritis. Am J Clin Nutr. 2000;71:349–51.
36. MacLean CH, Mojica WA, Morton SC, Pencharz J, Hasenfeld Garland R et al. Effects of omega-3 fatty acids on lipids and glycemic control in type II diabetes and the metabolic syndrome and on inflammatory bowel disease, rheumatoid arthritis, renal disease, systemic lupus erythematosus and osteoporosis. Evid Rep Technol Assess 2004; No 89, AHRQ Publ No 04-E012-2 Rockville MD: Agency Healthcare Res Qual.
37. Bartram HP, Gostner A, Scheppach W, Reddy BS, Rao CV, Dusel G, Richter F, Richter A, Kasper H. Effects of fish oil on rectal cell proliferation, mucosal fatty acid and prostaglandin E2 release in healthy subjects. Gastroenterology. 1993;105:1317–22.
38. Bartram HP, Gostner A, Scheppach W, Reddy BS, Rao CV, Dusel G, Richter F, Richter A, Kasper H. Missing antiproliferative effect of fish oil on rectal epithelium in healthy volunteers consuming a high fat diet. Potential role of the w3:w6 fatty acid ratio. Eur J Can Prev 1995; 4:231–237.
39. Calder PC, Deckelbaum RJ. Dietary fatty acids in health and disease: greater controversy, greater interest. Curr Opin Clin Nutr Metab Care. 2014;17:111–5.
40. Murphy RA, Mourtzakis M, Chu QS, et al. Supplementation with fish oil increases first line chemotherapy efficacy in patients with advanced non small cell lung cancer. Cancer. 2011;117:3774–80.
41. Roodhart JM, Daenen LG, Stigter EC, et al. Mesenchymal stem cells induce resistance to chemotherapy through the release of platinum-induced fatty acids. Cancer Cell. 2011;20:370–83.
42. Brasky TM, Darke AK, Song X et al Plasma phospholipids fatty acids and prostate cancer risk in the SELECT trial J Natl Cancer Inst 2013; 105:1132–1141.

43. Dahm CC, Gorst-Rasmussen A, Crowe FL, et al. Fatty acid patterns and risk of prostate cancer in a case—control study nested within the European Prospective Investigation into Cancer and Nutrition. Am J Clin Nutr. 2012;96:1354–61.
44. Chua ME, Sio MC, Sorongon MC, Dy JS. Relationship of dietary intake of omega-3 and omega-6 fatty acids with risk of prostate cancer development: a meta-analysis of prospective studies and review of literature. Prostate Cancer. 2012;2012:826254.
45. Anderson BM, Ma DW. Are all n-3 polyunsaturated acids created equal? Lipids Health Dis 2009; 10;8:33. doi:10.1186/1476-511x-8-33.
46. Carlson SE, Neuringer M. Polyunsaturated fatty acid status and neurodevelopment: a summary and clinical analysis of the literature. Lipids. 1999;34:171–8.
47. Descsi T, Koletzko B. Role of long-chain polyunsaturated fatty acids in early neurodevelopment. Nutr Neurosci. 2000;3:293–306.
48. Uauy R, Hoffman DR. Mena P et al Term infant studies of DHA and ARA supplementation on neurodevelopment: results of randomized controlled trials. J Pediatr. 2003;143:17–25.
49. Innis SM. Dietary omega 3 fatty acids and the developing brain. Brain Res. 2008;1237:35–43.
50. Hanebutt FL, Demmelmair H, Schiessl B, Larque E, Koletzko B. Long-chain polyunsaturated fatty acid transfer across the placenta. Clin Nutr. 2008;27:685–93.
51. Hibbeln JR, Davis JM, Steer C, Emmett P, Rogers I, Williams C, Golding J. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (ALSPAC Study): an observational cohort study. Lancet. 2007;369:578–85.
52. Birch EE, Garfield S, Castaneda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind, randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. Early Human Dev. 2007;83:279–84.
53. Koletzko B, Cetin I, Brenna JT for the Perinatal Lipid Intake Working Group. Dietary fat intakes for pregnant and lactating women: Consensus Statement. Br J Nutr 2007; 98:873–877.
54. Denomme J, Stark KD, Holub BJ. Directly quantitated dietary (n-3) fatty acid intake of pregnant Canadian women are lower than current dietary recommendations. J Nutr. 2005;132:206–11.
55. Lewis NM, Widga AC, Buck JS, Frederick AM. Survey of omega3 fatty acids in diets of Midwest low income pregnant women. J Agromed. 1995;2:49–57.
56. Houwelingen AC, Hornstra G. Relation between birth order and the maternal and neonatal DHA status. Eur J Clin Nutr. 1997;51:548–53.
57. Grandjean P, Bjerve KS, Weihe P, Stnerwald U. Birthweight in a fishing community: significance of essential fatty acids and marine food contaminants. Int J Epidemiol. 2001;30:1272–8.
58. Olsen SF, Secher NJ. Low consumption of seafood in early pregnancy as a risk factor for preterm delivery. BMJ. 2002;324(7335):447.
59. Szajewska H, Horvath A, Koletzko B. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. Am J Clin Nutr. 2006;83:1337–44.
60. Makrides M, Duley L, Olsen SF. Marine oil and other prostaglandin precursor supplementation for pregnancy, uncomplicated by pre-eclampsia or intrauterine growth restriction. Cochrane Database Syst Rev 2006; 3: CD003402.
61. Horvath A, Koletzko B, Szajewska H. Effect of n-3 long-chain polyunsaturated fatty acid supplementation of women in high -risk pregnancies on pregnancy outcomes and growth measures at birth: a meta-analysis of randomized controlled trials. Br J Nutr. 2007;98:253–9.
62. Jakobovich E. Granot E. Effect of DHA supplementation during pregnancy on birth weight and gestational length. Presented at the 4th World Congress of Pediatric Gastroenterology, Hepatology and Nutrition, Nov. 2012, Taipei, Taiwan (manuscript in preparation).
63. Xiang M, Harbige LS, Zetterstrom R. Long-chain polyunsaturated fatty acids in Chinese and Swedish mothers: diet, breast milk and infant growth. Acta Paediatr. 2005;94:1543–9.
64. Ruan C, Liu X, Man H, Ma X, Lu G, Duan G, DeFrancesco CA, Connor WE. Milk composition in women from five different regions of China: the great diversity of milk fatty acids. J Nutr. 1995;125:2993–8.
65. Brenna JT, Varamini B, Jensen RG, Diersen-Schade DA, Boettcher JA, Arterburn LM. Docosahexaenoic and arachidonic acid concentrations in human breast milk worldwide. Am J Clin Nutr. 2007;85:1457–64.
66. Larque E, Demmelmair H, Koletzko B. Perinatal supply and metabolism of long-chain polyunsaturated fatty acids: importance for the early development of the nervous system. Ann NY Acad Sci. 2002;967:299–310.
67. Bitman J, Wood L, Hamosh M, Hamosh P, Metha NR. Comparison of the lipid composition of breast milk from mothers of term and preterm infants. Amer J Clin Nutr. 1983;38:300–12.
68. Luukkainen P, Salo MK, Nikkari T. Changes in the fatty acid composition of preterm and term human milk from 1 week to 6 months of lactation. J Pediatr Gastroenterol Nutr. 1994;18:355–60.

69. Genzel-Boroviczeny O, Wahle J, Koletzko B. Fatty acid composition of human milk during the 1st month after term and preterm delivery. Eur J Pediatr. 1997;156:142–7.
70. Kovacs A, Funke S, Marosvolgyi T, Burus I, Decsi T. Fatty acids in early human milk after preterm and full term delivery. J Pediatr Gastroenterol Nutr. 2005;41:454–9.
71. Granot E, Ishay-Gigi K, Malaach L, Flidel-Rimon O. Human milk fatty acid composition after preterm and full term delivery. J Mater Fetal Neonatal Med 2015; (in press).
72. Koutras AK, Vigorita VJ. Fecal secretory immunoglobulin in breast milk versus formula feeding in early life. J Pediatr Gastroenterol Nutr. 1989;9:58–61.
73. Slade HB, Schwartz SA. Mucosal immunity: the immunology of breast milk. J Allergy Clin Immunol. 1987;80:348–58.
74. Kramer MS. Does breast feeding help protect against atopic disease? J Pediatr. 1988;112:181–90.
75. Pita ML, Fernandez MR. De-Lucchi C et al Changes in the fatty acid pattern of red blood cell phospholipids induced by type of milk, dietary nucleotide supplementation and postnatal age, in preterm infants. J Pediatr Gastroenterol Nutr. 1988;7:740–7.
76. Endres S, Ghorbani R. Kelley VE et al The effect of dietary supplementation with w3 polyunsaturated fatty acids on the synthesis of interleukin -1 and tumor necrosis factor by mononuclear cells. N Engl J Med. 1989;320:265–71.
77. Calder PC. Dietary fatty acids and lymphocyte functions. Proc Nutr Soc. 1998;57:487–502.
78. Granot E, Golan D, Berry EM. Breast–fed and formula-fed infants do not differ in immunocompetent cell cytokine production despite differences in cell membrane fatty acid composition. Am J Clin Nutr. 2000;72:1202–5.
79. Sherman MP. Human milk, fatty acids and the immune response: a new glimpse (Editorial). Am J Clin Nutr. 2000;72:1071–2.
80. Field CJ, Thomson CA, Van Aerde JE, et al. Lower proportion of CD45RO+ cells and deficient interleukin-10 production by formula-fed infants, compared with human-fed, is corrected with supplementation of long-chain polyunsaturated fatty acids. J Pediatr Gastroenterol Nutr. 2000;31:291–9.
81. Mazurak VC, Lien V, Field CJ, Goruk SD, Pramuk K, Clandinin MT. Long-chain polyunsaturated fat supplementation in children with low docosahexanoic acid intakes alters immune phenotypes compared with placebo. J Pediatr Gastroenterol Nutr. 2008;48:570–9.
82. Meydani SN. Effect of (n-3) polyunsaturated fatty acids on cytokine production and their biologic function. Nutrition. 1996;12:S8–14.
83. Saedisomeolia A, Wood LG, Garg ML, Gibson PG, Wark AB. Anti-inflammatory effects of long-chain n-3 PUFA in rhinovirus-infected cultured airway epithelial cells. Br J Nutr. 2009;101:533–40.
84. Vaisman N, Zaruk Y, Shirazi I, Kaysar N, Barak V. The effect of fish oil supplementation on cytokine production in children. Eur Cytokine Netw. 2005;16:194–8.
85. Granot E, Jakobovich E, Rabinowitz R, Levy P, Schlesinger M. DHA supplementation during pregnancy and lactation affects infants' cellular but not humoral immune response. Mediators Inflamm 2011:493925.
86. Burlingame B, Nishida C, Uauy R, Weisell R (eds). Fats and fatty acids in human nutrition (from the joint FAO/WHO expert consultation on fats and fatty acids in human nutrition). In: Annals of Nutrition and Metabolism, 2009; vol: 55, Karger AG, Basel.
87. Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, Cetin I, Decsi T, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J Perinat Med. 2008;36:5–14.
88. Michaelsen KF, Dewey KG, Perez-Exposito AB, Nurhasan M, Lauritzen L, Roos N. Food sources and intake of n-6 and n-3fatty acids in low-income countries with emphasis on infants, young children (6–12 months) and pregnant and lactating women. Mater Child Nutr 2011; Suppl 2:124–140.
89. Huffman SL, Harika RK, Eilander A, Osendarp SJ. Essential fats: how do they affect growth and development of infants and young children in developing countries? A literature review. Mater Child Nutr 2011; Suppl 3:44–65.
90. Kumwenda C, Dewey KG, Hemsworth J, Ashorn P, Maleta K, Haskell MJ. Lipid based nutrient supplements do not decrease breast milk intake of Malawian children. Am J Clin Nutr. 2014;99:617–23.
91. Briend A, Dewey KG. Complementary feeding: keeping the message simple. J Pediatr Gastroenterol Nutr. 2014;58:275.
92. Chaparro CM, Dewey KG. Use of lipid-based nutrient supplements (LNS) to improve the nutrient adequacy of general food distribution rations for vulnerable sub-groups in emergency settings. Matern Child Nutr. 2010;6 (Suppl 1):1–69.
93. Yang Z, Huffman SL. Modeling linoleic acid and alpha linolenic acid requirements for infants and young children in developing countries. Mater Child Nutr. 2013;9:S72–7.
94. Franco VH, Hotta JK, Jorge SM, Dos Santos JE. Plasma fatty acids in children with grade III protein malnutrition in its different forms: marasmus, marasmus kwashiorkor and kwashiorkor. J Trop Pediatr. 1999;45:71–5.
95. Decsi T, Koletzko B. Effects of protein-energy malnutrition and human immunodeficiency virus-1 infection on essential fatty acid metabolism in children. Nutrition. 2000;16:447–53.

96. Marin MC, Rey GE, Pedersoli LC, Rodrigo MA, Alaniz MJ. Dietary long-chain fatty acids and visual response in malnourished nursing infants. Prostagland Leukotrien Essent Fatty Acids. 2000;63:385–90.
97. Racine RA, Deckelbaum RJ. Sources of very- long-chain unsaturated omega-3 fatty acids: eicosapentanoic acid and docosahexanoic acid. Current Opin Clin Nutr Metab Care. 2007;10:123–8.
98. Gotz N, Bulbarello A, Konig-Grillo S, Dusterioh A, Volker M. Long-chain polyunsaturated omega-3 fatty acids in food development. Sight Life. 2013;27:12–7.